The Preventive Effect of Vitamin E on Gallstone Formation (2)

A Study of the Prevention of Gallstone Formation and Protection from Liver Disorder in Hamsters

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The Preventive Effect of Vitamin E on Gallstone Formation
(2) A Study of the Prevention of Gallstone Formation
and Protection from Liver Disorder in Hamsters

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Summary

A lithogenic diet causes a decrease of the (TBA/CH) ratio in the bile. Hamsters fed a
lithogenic diet were administered either an a-tocopherol solution including polyethylene glycol
and hydrogenetic caster oil, or these solvents without a-tocopherol. a-Tocopherol produced
a decrease in cholesterol, an increase in TBA, and a reduction of lithogenicity, but the solvents
had no marked effect.

a-Tocopherol remarkably reduced the incidence of gallstone formation, and a definite
inverse correlation was observed between a-tocopherol in the liver and the lithogenic index of
hepatic or gallbladder bile. The lithogenic diet raised serum total cholesterol, TG, and PL
level, but a-tocopherol remarkably reduced total cholesterol, TG, VLDL-cholesterol, LDL-TG,
VLDL-TG, and VLDL-PL levels.

Liver disorders were induced by the continuous addition of 0.5% CDCA to a lithogenic diet.
a-Tocopherol increased bile flow, bile acid and phospholipid output, and ameliorated deterio-
rations in liver function, as measured by GOT and GPT values. Furthermore, it lowered the
increased serum TG and total cholesterol levels.

Introduction

It was previously confirmed that a-tocopherol produces a decrease in biliary cholesterol,
an increase in GCDCA, and a corresponding improvement of cholesterol solubilizing capacity
(TBA/CH) in the bile of hamsters fed a chow diet. The present studies were conducted to show
the effect of a-tocopherol on the biliary lipids in hamsters with lithogenous bile induced by a
lithogenic diet.

Materials and Methods

Experiment I

Twenty-one male golden hamsters (five weeks old, weighing 62 to 82 g) purchased from the
Shizuoka Laboratory Animal Center were fed a lithogenic diet for five weeks. All animals were housed individually in suspended wire-mesh cages in an environmentally controlled room (26°C). Hamsters were divided into three groups, Group I (n=6) which was a control group; Group II (n=7) consisting of hamsters administered a solution of 140 mg of polyethylene glycol 400 (PG) and 200 mg of hydrogenetic castor oil 60 (HCO); and Group III (n=8) consisting of hamsters administered a solution containing 100 mg of α-tocopherol per 2 ml (in addition to PG and HCO). These solutions were administered intraperitoneally for four consecutive days on the fifth week at a dosage of 20 mg/kg body weight per day.

The lithogenic diet contained 60% glucose, 20% milk casein, 10% butter, 3.5% carboxy methyl cellulose, 5% salt mixture, 0.5% choline chloride and 1% vitamin mixture (Panvitan®). Appropriate fresh diets were prepared and replaced daily in the cages. Blood was taken from the abdominal aorta and gallbladder bile was aspirated by the insulin injector “Myjector”. A few stones were recorded as (+), stones occupying less than half of the lumen were recorded as (2+), and sufficient stones to occupy more than half of the lumen were recorded as (3+). Measurements were made of liver weight, biliary cholesterol and phospholipids, individual bile acids in the bile, serum cholesterol, and serum and liver tocopherol concentrations. Cholesterol and phospholipids were measured by enzymatic methods [7, 20], and individual bile acid concentrations were evaluated by the HPLC method [21]. Liver and serum tocopherol concentrations were determined by the ABE method [11]. The lithogenic Index (LI) was calculated using the formula of Thomas and Hofmann based on the limits of cholesterol solubility defined by Small and Admirand [27]. All the results were shown as “Mean±SEM”, and the Student’s t-test was utilized to make statistical comparisons.

Experiment II

Thirty hamsters, five weeks old, weighing 53 to 80 g were fed the lithogenic diet for three weeks. In Group IV, 16 hamsters received dl-α-tocopheryl acetate five times at a dosage of 20 mg/kg. The first administration occurred before feeding with the lithogenic diet and was also given on the 7th, 9th, 12th and 15th day. Group V was a control group. On the 21st day, the animals were sacrificed. The numbers of cholesterol gallstones in their gallbladders were identified, and the cystic duct was then ligated. A polyethylene tube with 0.28 mm ID (Intramedic, PE-10, Clay Adams, USA) was inserted in the common bile duct and hepatic bile was collected for an hour. Bile flow and serum lipoprotein concentration were investigated. The incidence of gallstone formation in every group was compared using the χ² test.

Experiment III

Forty-eight hamsters, six weeks old, weighing 73 to 100 g were fed the lithogenic diet containing 0.5% CDCA. In Group VI, 26 hamsters received dl-α-tocopheryl acetate four times at a dosage of 20 mg/kg. The first administration occurred before feeding with the lithogenic diet, and other administrations, on the first, 13th and 15th day. Bile flow and liver function tests were investigated. The remaining 22 hamsters were used as a control group (Group VII).
Results

The mean intake of the experimental diet was 7.5 g daily in all groups.

(1) Incidence of gallstone formation

Only white stones and mixed white-yellow stones were considered as cholesterol gallstones, and black (pigment) stones were excluded from the count of gallstones. All hamsters except one hamster in Group II had gallstones in Experiment I. Gallstones were found 86% in Group V and 44% in Group IV (Table 1). Gallstones were found 90.9% in Group VII and 61.5% in Group VI. Thus, α-tocopherol significantly reduced the incidence of gallstone formation (p<0.05).

(2) Serum and liver α-tocopherol concentrations

The animals receiving α-tocopherol showed a 4-fold increase in serum and liver α-tocopherol concentrations, whereas those in Group II were similar to the animals in Group I (Fig. 1). α-Tocopherol administration caused a 3-fold increase of serum and liver α-tocopherol concentrations.

Table 1. Incidence of experimental gallstone formation

<table>
<thead>
<tr>
<th>Group</th>
<th>(-)</th>
<th>(+)</th>
<th>(#)</th>
<th>(##)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14%</td>
<td>7%</td>
<td>50%</td>
<td>29%</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>56%*</td>
<td>19%</td>
<td>25%</td>
<td>0%</td>
</tr>
</tbody>
</table>

* P<0.05

Fig. 1. Serum and liver α-tocopherol concentrations
in Experiment II, and it caused a 2.5-fold increase in serum α-tocopherol concentration, and also a 5-fold increase in liver α-tocopherol concentration in Experiment III.

(3) Serum lipids and lipoprotein level

A slight decrease in serum cholesterol level was observed in Groups II and III. The lithogenic diet raised serum cholesterol and the TG level, and α-tocopherol produced a marked diminution of total cholesterol and TG level (P<0.01, Table 2). Although α-tocopherol administration produced no significant changes in PL, HDL-cholesterol, LDL-cholesterol, HDL-TG, HDL-PL and LDL-PL, α-tocopherol did significantly reduce VLDL-TG and VLDL-cholesterol in Experiment II. The lithogenic diet plus CDCA supplement raised serum cholesterol and the TG level. Although α-tocopherol normalized the increased cholesterol level, TG remained at high levels (Table 3). CDCA caused a significant decrease in HDL cholesterol level, but α-tocopherol did not decrease HDL cholesterol level.

Table 2. Serum lipids and lipoprotein levels in hamsters of Experiment II

<table>
<thead>
<tr>
<th></th>
<th>control (n=5)</th>
<th>α-tocopherol (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>324 ± 45</td>
<td>185 ± 14</td>
</tr>
<tr>
<td>TG*</td>
<td>1179 ± 191</td>
<td>459 ± 130</td>
</tr>
<tr>
<td>PL</td>
<td>715 ± 117</td>
<td>376 ± 27</td>
</tr>
<tr>
<td>HDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>110 ± 11</td>
<td>105 ± 4</td>
</tr>
<tr>
<td>TG*</td>
<td>126 ± 18</td>
<td>118 ± 25</td>
</tr>
<tr>
<td>PL</td>
<td>264 ± 13</td>
<td>256 ± 6</td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>32 ± 5</td>
<td>37 ± 8</td>
</tr>
<tr>
<td>TG*</td>
<td>55 ± 13</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>PL</td>
<td>42 ± 9</td>
<td>31 ± 7</td>
</tr>
<tr>
<td>VLDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>183 ± 36</td>
<td>43 ± 11</td>
</tr>
<tr>
<td>TG*</td>
<td>998 ± 164</td>
<td>313 ± 120</td>
</tr>
<tr>
<td>PL*</td>
<td>409 ± 111</td>
<td>90 ± 20</td>
</tr>
</tbody>
</table>

*: P<0.01, **: P<0.05

Table 3. Serum lipids level in hamsters of Experiment III

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum lipids (mg/dl ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total cholesterol</td>
</tr>
<tr>
<td>IX (n=3)</td>
<td>150 ± 7.0</td>
</tr>
<tr>
<td>VIII (n=3)</td>
<td>163 ± 17.2</td>
</tr>
<tr>
<td>VII (n=14)</td>
<td>178 ± 11.0</td>
</tr>
<tr>
<td>VI (n=16)</td>
<td>151 ± 12.6</td>
</tr>
</tbody>
</table>
Table 4. Concentration of biliary lipids in hamsters of Experiment I

<table>
<thead>
<tr>
<th>Group</th>
<th>Total bile acids (μmol/ml ± SEM)</th>
<th>Cholesterol</th>
<th>Phospholipids</th>
<th>TBA/CH</th>
<th>Lithogenic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>32.5 ± 4.8 (78.6 ± 1.1%)</td>
<td>4.3 ± 0.6 (10.5 ± 0.5%)</td>
<td>4.2 ± 0.3 (10.9 ± 1.1%)</td>
<td>7.6 ± 0.4</td>
<td>1.24 ± 0.07</td>
</tr>
<tr>
<td>II</td>
<td>32.5 ± 5.0 (78.3 ± 3.1%)</td>
<td>4.2 ± 0.7 (10.3 ± 1.0%)</td>
<td>4.1 ± 0.5 (11.4 ± 2.7%)</td>
<td>8.1 ± 1.0</td>
<td>1.24 ± 0.10</td>
</tr>
<tr>
<td>III</td>
<td>39.3 ± 1.8 (85.4 ± 1.0%)</td>
<td>3.0 ± 0.3 (6.4 ± 0.6%)</td>
<td>3.8 ± 0.3 (8.2 ± 0.5%)</td>
<td>14.0 ± 1.4</td>
<td>0.82 ± 0.06</td>
</tr>
</tbody>
</table>

(4) Biliary cholesterol and phospholipid level

The animals receiving α-tocopherol showed a lower percentage of biliary cholesterol, but no significant differences were observed in the percentage of biliary phospholipids in Experiment I (Table 4). α-Tocopherol significantly reduced the cholesterol level in hepatic bile (0.85 ± 0.11 μmol/ml vs 1.41 ± 0.12 μmol/ml, p<0.01). The mean cholesterol concentration in hamsters without gallstones in Group IV (the NG group) was lower than in hamsters with gallstones in Group IV (the GS group). α-Tocopherol also reduced cholesterol level in gallbladder bile (p<0.001). The value in the NG group (3.1 ± 0.6%) was significantly lower than in the GS group (5.5 ± 0.8%, p<0.05). No significant differences in phospholipid concentration in hepatic and gallbladder bile were observed among these groups. In the animals receiving α-tocopherol (Group VI), there was a slight decrease of the cholesterol level in bile. There was no significant difference in phospholipid concentration between Groups VI and VII.

(5) The composition and concentrations of bile acids in bile

The animals receiving α-tocopherol showed a greater percentage of total bile acid due to the increase of GCDCA, compared with Groups I and II (Table 4). On the other hand, no significant difference was observed in the percentage of taurine-conjugated or unconjugated bile acids among three groups (Table 5). The lithogenic index in animals receiving α-tocopherol was

Table 5. Composition and concentration of bile acids in hamsters of Experiment I

<table>
<thead>
<tr>
<th>Group</th>
<th>Glycine conjugated</th>
<th>Taurine conjugated</th>
<th>Unconjugated</th>
<th>GCDCA</th>
<th>GCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>20.8 ± 3.0 (64.3 ± 0.5%)</td>
<td>11.1 ± 1.6 (33.5 ± 0.8%)</td>
<td>0.7 ± 0.2 (2.2 ± 0.5%)</td>
<td>16.1 ± 2.5 (49.3 ± 0.9%)</td>
<td>4.6 ± 0.5 (14.8 ± 0.8%)</td>
</tr>
<tr>
<td>II</td>
<td>20.5 ± 3.0 (63.4 ± 0.7%)</td>
<td>11.4 ± 1.9 (34.8 ± 0.8%)</td>
<td>0.6 ± 0.2 (1.8 ± 0.4%)</td>
<td>15.9 ± 2.6 (48.8 ± 1.5%)</td>
<td>4.6 ± 0.6 (14.6 ± 1.8%)</td>
</tr>
<tr>
<td>III</td>
<td>25.7 ± 1.3 (65.4 ± 0.9%)</td>
<td>13.1 ± 0.7 (33.3 ± 0.9%)</td>
<td>0.5 ± 0.1 (1.2 ± 0.2%)</td>
<td>20.6 ± 1.1 (52.2 ± 1.2%)</td>
<td>5.2 ± 0.3 (13.2 ± 0.7%)</td>
</tr>
</tbody>
</table>
Fig. 2. TBA/CH ratio and lithogenic index in hamsters of Experiment I

![Graph of TBA/CH ratio and lithogenic index](image)

Fig. 3. Correlation between α-tocopherol concentration in the liver and lithogenic index of the bile in hamsters of Experiment I

![Graph showing correlation](image)

significant lower than in the other two groups (Fig. 2). No significant differences in TBA/CH ratio and lithogenic index were observed between Group I and Group II. A definite inverse correlation was observed between α-tocopherol concentration in the liver and the lithogenic index of the bile \((r= -0.804\); Fig. 3).

Hepatic bile in the biliary bile acid composition of the lithogenic controls (Group V) indicated that CDCA was the major bile acid (53.9%) and that CA accounted for 33.2%. In the animals receiving α-tocopherol, there was a marked increase in biliary CDCA of 62.2%, while CA decreased. Glycine-conjugated bile acid concentration in Group IV was significantly higher than in the control group, because α-tocopherol produced a marked increase of GCDCA compared
Table 6. Composition of bile acid in hamsters of Experiment II

<table>
<thead>
<tr>
<th>Group</th>
<th>GCDCCA (g)</th>
<th>GCA (g)</th>
<th>TCDCCA (g)</th>
<th>TCA (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V (n=11)</td>
<td>40.6 ± 3.6%</td>
<td>26.0 ± 2.6%</td>
<td>12.1 ± 2.4%</td>
<td>6.7 ± 1.7%</td>
</tr>
<tr>
<td>IV (n=13)</td>
<td>27.8 ± 4.0%</td>
<td>30.8 ± 3.2%</td>
<td>10.9 ± 2.1%</td>
<td>7.2 ± 1.3%</td>
</tr>
</tbody>
</table>

Table 7. Concentration of biliary lipids and lithogenic index of hepatic bile in hamsters of Experiment II

<table>
<thead>
<tr>
<th>Group</th>
<th>Biliary lipids (nmol/ml ± SEM)</th>
<th>Lithogenic index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total bile acid</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>V (n=11)</td>
<td>9.0 ± 1.5</td>
<td>1.41 ± 0.12</td>
</tr>
<tr>
<td>IV (n=13)</td>
<td>9.9 ± 0.5</td>
<td>0.85 ± 0.11</td>
</tr>
</tbody>
</table>

Table 8. Concentration of biliary lipids and lithogenic index of gallbladder bile in hamsters of Experiment II

<table>
<thead>
<tr>
<th>Group</th>
<th>Biliary lipids (nmol/ml ± SEM)</th>
<th>Lithogenic index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total bile acid</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>V (n=11)</td>
<td>19.7 ± 2.7</td>
<td>3.46 ± 0.42</td>
</tr>
<tr>
<td>IV (n=16)</td>
<td>58.6 ± 8.9</td>
<td>2.15 ± 0.18</td>
</tr>
</tbody>
</table>

with Group V (p<0.05; Table 6). The lithogenic index in Group IV was significantly lower than in the control group (Table 7). The mean TBA in the NG group was significantly higher than in Group V (p<0.05), while that in the GS group was nearly equal to that of the control group. Lithogenic index in the NG group was lower than in the GS group.

The animals receiving α-tocopherol showed a significantly greater percentage of total bile acid than did the control group (Table 8), and a greater percentage of GCDCCA than did Group V (p<0.05) in the gallbladder bile. The lithogenic index, 0.51 in Group IV, was significantly lower than the 1.39 in Group V (p<0.05). The increase in liver α-tocopherol concentration was in inverse correlation with the lithogenic index in the range of 66.1 μg/g to 264.0 μg/g (r= -0.614; Fig. 4).

Total bile acids concentration in the NG subgroup of Group VI was significantly higher than the GS subgroup of Group VI or Group VII (Table 9). The bile acid composition of the
CDCA controls (Group VII) indicated that CDCA was the major bile acid (86.3%), but no significant difference was observed in the percentage of CDCA between Groups VI and VII. The lithogenic index in the NG group was significantly lower than in Group VII, but no significant difference was seen between hamsters with gallstones in Groups VI and VII.

(6) Bile flow and output of biliary lipids

α-Tocopherol administration caused a slight increase in bile flow, but the difference was not significant. No significant differences in cholesterol, phospholipids, and TBA output were observed between Groups IV and V. α-Tocopherol administration produced an increase in bile flow, the hepatic output of bile acids (88.2±10.8 vs 44.7±5.2 μmol/hr, P<0.001; Fig. 5), and the output of biliary phospholipids. Cholesterol output in hamsters receiving α-tocopherol (Group VI) was slightly higher than in Group VII, despite the decreased cholesterol level observed in hamsters of the Group VI. There was no difference in the secretion of these biliary lipids between the NG and GS groups of Group VI. Liver α-tocopherol concentration did not correlate with the lithogenic index of the bile (r=−0.046).
(7) Liver function test

The CDCA supplement (Group VII) caused a significant increase of GOT and GPT values, while the lithogenic diet (Group VIII) had no influence on these enzyme activity compared to the chow diet group animals (Group IX). α-Tocopherol improved these impaired values (Fig. 6), but they remained at high levels.

Discussion

The hamster has the propensity to produce lithogenous bile when fed on one of a variety of alimentary manipulations, such as that rich in glucose but fat free or containing high glucose.
and butter-fat, and that containing 2.4 mg cholesterol/g of diet and 15 µg ethinyl estradiol. For the reasons that we neither consume such a large amount of cholesterol nor live on a fat-free diet every day, the lithogenic diet of Tanamura was selected as the model in the present study.

It is well documented that a dramatic increase in hepatic cholesterol synthesis (an increase of HMG-CoA reductase activity) in hamsters fed on the lithogenic diet can be observed from the second day of feeding, and also that the size of gallstones develops in the 4th week, while they are still small in the 3rd week. On the other hand, it is generally accepted that gallstones in hamsters can not be dissolved even if CDCA or UDCA is administered. Thus, it was decided to administer α-tocopherol on four consecutive days, to evaluate its effects on the biliary lipids and to compare its effects with those of the solvents. In Groups IV and VI, α-tocopherol was administered every five days, because while required only six hours to stimulate some enzyme activity in the liver, it took more than four days to exert effects on the biliary lipids.

From the results, PG and HCO appeared to have no effect on the biliary lipids, and it was the α-tocopherol itself that improved the lithogenic index. α-Tocopherol prevented gallstone formation in half of the animals, but its efficacy in Group VI was worse than in Group IV. From the fact that the liver α-tocopherol concentration correlated well with the lithogenic index, and that liver and serum α-tocopherol concentrations in Group VI were lower than those in Group IV, it was assumed that some part of α-tocopherol was consumed in protecting the liver cells from the intoxication by CDCA, and thus the efficacy of the prevention remained at a low level. It is well known that α-tocopherol is consumed in protecting cell membranes from peroxide, and Ogasawara demonstrated that α-tocopherol was consumed when it was mixed with liver cells pre-treated with peroxide. The CDCA supplement caused a marked increase of the CDCA fraction, and the increase of CDCA was usually favorable to improve the cholesterol solubilizing capacity in the bile. However, when the amount of CDCA in the administered diet was more than 0.1%, it raised the incidence of gallstone formation in hamsters. As was observed in the present study, there was no correlation between the lithogenic index and CDCA concentration in the bile.

There was a definite inverse correlation between the liver α-tocopherol concentration and the lithogenic index of the bile (r = -0.804), and a moderate correlation (r = -0.614) was observed within the range of 66 to 264 µg/g of liver α-tocopherol. Since the mean liver α-tocopherol concentration in the lithogenic controls (52.7 µg/g) was lower than 66.1 µg/g, it was confirmed that the amount of α-tocopherol by ordinary dietary intake was not enough to show its effects on the biliary lipids.

The present study was the first to evaluate the serum lipoprotein level in hamsters receiving α-tocopherol. It was confirmed that this lithogenic diet remarkably raised serum total cholesterol and TG levels, and that α-tocopherol produced a significant diminution of serum total cholesterol and TG level. α-Tocopherol significantly reduced VLDL-cholesterol, VLDL-TG, and VLDL-PL. Kamata and Ginsberg investigated the hepatic HMG-CoA reductase activity in hamsters fed a lithogenic diet, and found a significant increase in this enzyme activity.
On the other hand, in the present study a marked increase of serum VLDL, especially VLDL-TG was observed in hamsters fed on the same diet. This result is consistent with the view that VLDL-TG synthesis correlated well with the activity of HMG-CoA reductase in the liver\(^9\). From the result that \(\alpha\)-tocopherol caused a decrease of VLDL-TG, it may be suggested that \(\alpha\)-tocopherol suppressed hepatic HMG-CoA reductase activity.

On the effect of \(\alpha\)-tocopherol on hepatic cholesterol 7a-hydroxylase, there were no investigation in the animals receiving \(\alpha\)-tocopherol. In the prior studies, the result suggesting an increased activity of this enzyme was obtained in hamsters receiving \(\alpha\)-tocopherol. SUGANO\(^{23}\) indicated that the reduced HMG-CoA reductase activity induced an increased hepatic cholesterol 7a-hydroxylase activity in rats. The reduced HMG-CoA reductase activity by \(\alpha\)-tocopherol might increase cholesterol 7a-hydroxylase activity in hamsters. From the fact that \(\alpha\)-tocopherol caused a decrease in biliary cholesterol and an increase in GCDCA in hamsters with a lithogenous bile, the activity of cholesterol 7a-hydroxylase may be accelerated. Stillmore, from the facts that \(\alpha\)-tocopherol caused a marked increase of GCDCA, and a decrease in biliary cholesterol in older hamsters\(^{21}\) whose cholesterol 7a-hydroxylase activity was said to be decreased\(^{24}\), suggested that \(\alpha\)-tocopherol may directly accelerate this enzyme activity.

The effect of \(\alpha\)-tocopherol on cholesterol 12a-hydroxylase activity must be argued. The activity of this enzyme in patients with cholesterol gallstones is usually suppressed\(^{22,29}\) and its activity in these hamsters with lithogenous bile, especially in the male hamsters whose major bile acid is CA\(^{13}\) was also considered to be decreased. In fact, the finding that CDCA could prevent gallstone formation in spite of the suppression of 12a-hydroxylase activity\(^{14}\), suggests that the change of this enzyme may be of little value. In our study, although \(\alpha\)-tocopherol caused an increase of CA concentration in the bile, the percentage of the CA fraction actually decreased, while that of the CDCA fraction markedly increased.

In the therapeutic dissolution of gallstones, high dosages of CDCA have been highly effective, but a corresponding increase in the incidence of liver disorders such as increased serum transaminase (30 to 51\%) have been observed\(^8,8,10,23\). In Experiment III, it was confirmed that \(\alpha\)-tocopherol could ameliorate the impairment of liver function by CDCA. In a study by OKUN\(^{17}\) on the toxicity of CDCA, LD50 was 6275 mg/kg of the diet in male hamsters. Thus, 5000 mg/kg of diet (498~682 mg/kg body weight) of CDCA was administered to hamsters every day in Groups VI and VII. No hamsters expired, and they exhibited no changes in behavior, appearance, and food consumption. Blood chemistry evaluations, however, indicated marked increases in serum GPT and GOT in all hamsters receiving CDCA. Since the capacity of sulfate conjugation of CDCA in hamsters is small\(^{28}\), CDCA administration easily caused an increase of serum transaminase. 20 mg/kg body weight of \(\alpha\)-tocopherol ameliorated the impaired GOT and GPT values to some extent. From this result, if \(\alpha\)-tocopherol is administered concurrently with CDCA in clinical cases, the increase of serum transaminase seems to be prevented. From the above results, clinical applications would be expected to promote the dissolution of gallstones and prevent the side effects of CDCA.
The author wishes to express their sincere gratitude to Professor KAZUE OZAWA, Assistant Professor HIROSHI TANIMURA, and Professor YORINORI HIKASA, Second Department of Surgery, Kyoto University, for their overall instruction in the course of this work. This work was in part supported by Scientific Grant-in-Aid No. 59570571 for Scientific Research from the Japan Ministry of Education, Science and Culture. A part of the present study was presented at the International Bile Acid Meeting, Tokyo, 1986, at the 71th General Meeting of the Japanese Society of Gastroenterology, 1984, and at the 37th General Meeting of the Vitamin Society of Japan, 1985.

References


ビタミンEの胆石形成予防効果に関する研究

(2) α-Tocopherolの胆石形成予防効果および肝機能障害抑制効果に関する研究

京都大学医学部第2外科学教室（指導：小澤和恵教授）

斎藤 徹

α-tocopherolと飽和脂肪酸を主とした実験的胆石形成食にて飼育したハムスターにα-tocopherolを20mg/kg体重、4回腹腔内投与し、胆石形成予防効果を検討し、以下の成績を得た。

1. 今回使用した実験的胆石形成食は、すでに共同研究者である谷村らにより肝臓でHMG-CoA Reductase活性を亢進させることにより、胆汁中にコレステロール濃度を上昇させ、逆に胆汁中総胆汁酸濃度を減少させ、催石指数を高め、その結果ハムスターの胆囊内に4～6週間にて胆石を形成することが知られている。この実験的胆石形成食飼育中のハムスターにα-tocopherolを投与すると、胆汁中コレステロール濃度を低下させ、胆汁中胆汁酸のうちGCDCA濃度を上昇させ、それに基づく催石指数の改善により、コレステロール胆石の形成を1/2以下に予防することが明らかにされた。

2. また、作用発現時のα-tocopherol濃度は血清濃度にて通常の2倍に相当する20μg/mlに達していたことが必要で、肝臓内のα-tocopherol濃度と血清α-tocopherol濃度はよく相関し、肝臓内のα-tocopherol濃度と胆汁の催石指数がr=−0.614にてよく相関することを明らかにした。

3. CDCA負荷により肝機能障害を作成した動物においてもα-tocopherolの投与は胆汁中コレステロール濃度の低下と、胆汁中GCDCA濃度の上昇、それに基づく催石指数の改善を来し、コレステロール胆石の形成を有意に予防するとともに、GOTやGPTなど肝機能検査値の上昇を抑制した。

4. 血清脂質に及ぼすα-tocopherol投与の影響は、実験的胆石形成食による血清総コレステロール、TG、VLDL-TGの上昇を改善することを明らかにした。

以上の実験成績からα-tocopherolの適切な投与量により、肝臓内α-tocopherol濃度を上昇させることができれば、胆石形成を予防しうるとともに、CDCA肝機能障害の抑制、さらに血清脂質の改善効果をも有することが明らかにし、この領域における臨床応用が期待される。